Expression of Basic Fibroblast Growth Factor (bFGF) in Kaposi's Sarcoma: An Immunohistologic Study

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Ten cases of Kaposi's sarcoma (KS) including five AIDS-KS, one classical KS, and four pseudo-KS (acroangiodermatitis) were investigated for their expression of basic fibroblast growth factor. Antigen expression was demonstrated by immunoperoxidase staining of cryostat sections with affinity-purified anti-bFGF antibodies.

It was found that bFGF was strongly expressed in basal and suprabasal keratinocytes, which were also intensively stained in normal skin biopsies. The growth factor was generally absent from the endothelial cells and spindle cells of the neoplasms. These cell types exhibited a very faint staining in a small number of lesions.

The studies provide strong evidence that proliferation of KS tumor cells may not be explained by autocrine secretion mechanism of the growth factor, which has been suggested in previous reports with in vitro cultured KS cell lines. *J Invest Dermatol 95:238–240, 1990*

Kaposi's sarcoma (KS) was first described more than 100 years ago as a rare disease affecting predominantly elderly men of Caucasian ancestry [1]. During the last years, a dramatic change has occurred in the epidemiology due to the association of Kaposi's sarcoma with acquired immuno-deficiency syndrome.

The neoplasm is a multifocal lesion of unclear histogenesis and unknown pathogenesis. It reveals a complex histologic pattern characterized by the presence of irregular vascular elements and proliferative spindle-shaped structures. The former have been identified as endothelial cells based on the presence of Weibel-Palade bodies and von-Willebrand factor. There is no general agreement about the origin of the spindle cell component (reviewed in [2,3]). Most recent evidence in which lesions were analyzed by immuno- and enzyme-histochemical comparison favors a histogenesis of Kaposi's sarcoma cells from vascular endothelium [4,5].

Besides the unclear cellular origin, the pathogenic mechanisms leading to Kaposi's sarcoma are totally unknown. The incidence of Kaposi's sarcoma in patients under immunosuppressive therapy [6] and the absence of retroviral DNA sequences in AIDS-lesions [7] argues against a direct viral etiology of Kaposi's sarcoma. The unrestricted growth of Kaposi's sarcoma cells was explained hypothetically either by viral-dependent protooncogene activation or by mechanisms in which abnormal production of autocrine or paracrine growth factors lead to cell proliferation. Delli Bovi et al [8] isolated a KS-derived oncogene called hst/k-fgf whose product has homology to the fibroblast growth factors. Amplifications or rearrangements of certain oncogenes, however, which are generally believed to be involved in tumorigenesis cannot be observed in Kaposi's sarcoma [9].

Recent investigations of Ensoli et al [10] suggest that proliferation of KS cells may be caused by the autocrine production and high-level expression of basic fibroblast growth factor (bFGF) and interleukin 1. As these data were elaborated from in vitro cultured cells that may not necessarily correlate to in vivo situations, it was the aim of our study to examine the in situ expression of bFGF using the indirect immunoperoxidase technique.

MATERIALS AND METHODS

Antibodies Polyclonal antibodies against bFGF were kindly provided by Dr. W. Risau (Max-Planck-Institute of Psychiatry, Martinsried, FRG). The antibodies were raised in rabbits using recombinant human bFGF for immunization [11]. They specifically detect bFGF but not acidic FGF in Western blots at a 1000-times-higher dilution of the antisera (Risau et al, manuscript in preparation). For immunohistology, the antibodies were purified by affinity chromatography over bFGF-coupled sepharose.

Polyclonal antibodies against factor VIII–related antigen were purchased from Dakopatts (Denmark).

Immunohistology Immunostaining on cryostat sections was essentially performed as described [12]. Briefly, cryostat sections were air dried, fixed in acetone, and preincubated with 50% normal goat serum (NGS) in PBS. After 30 min, anti-bFGF antibodies (1.5 μg/ml, diluted in 10% NGS) were incubated for 90 min. Bound antibodies were detected with goat–anti-rabbit IgG–peroxidase conjugate (Dianova, Hamburg, FRG) followed by development with 3-amino-4-ethylcarbazole. Sections were counterstained with Mayer's hemalaun and embedded in Aquamount.

Controls were performed either by a) replacing the anti-bFGF IgG with corresponding amounts of normal rabbit IgG or b) adsorption of anti-bFGF IgG with a tenfold excess of recombinant human bFGF prior to the incubation.

RESULTS

Cryostat sections of 10 cases of Kaposi's sarcoma including five AIDS-KS, one classical KS, and four pseudo-KS (acroangiodermatitis) were examined. All lesions were restricted to the skin except one of classical KS, which was taken from the oral mucosa. The

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Abbreviations:
AIDS: acquired immuno-deficiency syndrome
bFGF: basic fibroblast growth factor
IgG: immunoglobulin G
KS: Kaposi's sarcoma
NGS: normal goat serum
PBS: phosphate-buffered saline

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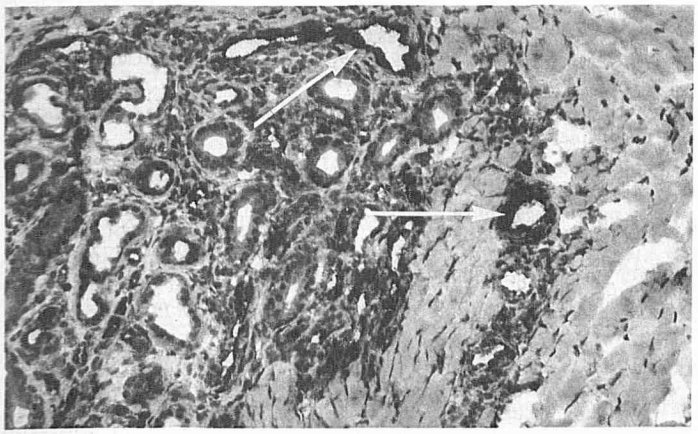

Figure 1. Light micrograph of factor VIII expression in AIDS-KS. Staining was performed with a polyclonal antiserum against factor VIII–related antigen. Sections were counterstained with Mayer's hemalaun. Factor VIII is expressed in nearly all blood vessels (*arrows,* magnification ×150).

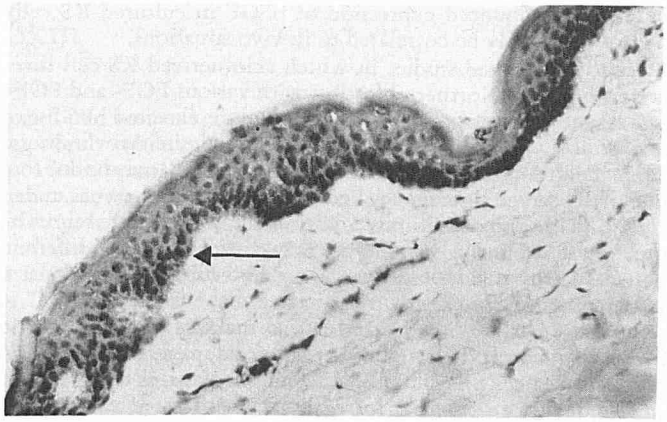

Figure 3. Staining of bFGF in normal skin. Expression of bFGF is also exclusively found in keratinocytes (*arrow,* magnification ×180).

sections all revealed a typical histologic pattern of irregular vascular elements lined with pleiomorphic endothelial cells and spindle-shaped cell structures. In most cases, extravasation of erythrocytes and hemosiderin deposition was observed. In three biopsies of pseudo-KS and one of AIDS-KS no spindle cells were found.

Factor VIII staining was strongest in non-neoplastic cells of small capillaries, arterioles, and veins, whereas poorly differentiated endothelial cells were less intensely stained (Fig 1). Spindle cells were always negatively stained, consistent with the observations of other investigators [4,5].

Staining with anti-bFGF antibodies revealed that expression of the growth factor was mainly restricted to the basal and suprabasal layer of the epidermis, where keratinocytes showed a large accumulation of bFGF (Fig 2). Staining was clearly specific, because controls using non-immune IgG or adsorption of the antiserum against recombinant bFGF abolished the staining. Besides strongly stained keratinocytes, other cell types such as vascular or spindle-shaped cells were largely devoid of bFGF. These cell types also remained unlabeled with higher concentrations (up to 10 μg/ml) of the antibodies. Some biopsies (two of pseudo-KS and one of AIDS-KS) additionally exhibited a faint staining of endothelial and spindle cells, whereby mainly more differentiated endothelial cells weakly expressed bFGF. Staining, however, was only found in single scattered cells. There were no differences in the staining reactivity between the various forms of Kaposi's sarcoma and normal skin biopsies (Fig 3).

Due to the negative staining of the hyper- and neoplastic cells observed in Kaposi's sarcoma we further analyzed bFGF expression in other kinds of vascular proliferations such as granuloma pyogenicum (four), hemangiosarcoma (two), and capillary hemangioma (five). Although also in the first two lesions keratinocytes were the exclusively stained cell type, hemangioma exhibited an additional staining of neoplastic vascular cells with a more pronounced reaction in differentiated tumor cells (Fig 4).

DISCUSSION

The recent findings of Ensoli et al [10] that Kaposi's sarcoma–derived cell lines are characterized by a pronounced expression of basic fibroblast growth factor and interleukin 1 prompted us to investigate the presence of bFGF in situ using the indirect immunoperoxidase technique. In contrast to these authors, we do not find a pronounced expression of the growth factor in neoplastic KS cells. Staining of KS lesions was not altered in comparison to normal skin. In both tissues, bFGF seemed to be confined to the epidermal cells where large amounts were accumulated, especially in the basal and suprabasal keratinocytes. Other cell types generally did not exhibit any detectable expression of the growth factor. Faint staining was only observed in individual endothelial cells or single scattered tumor cells of some lesions. This is particularly striking because we have previously shown [13] that bFGF is largely enhanced in other skin tumors such as melanoma and basal cell carcinoma and other neoplasms as, e.g., osteosarcoma or gastric and mamma carcinoma.

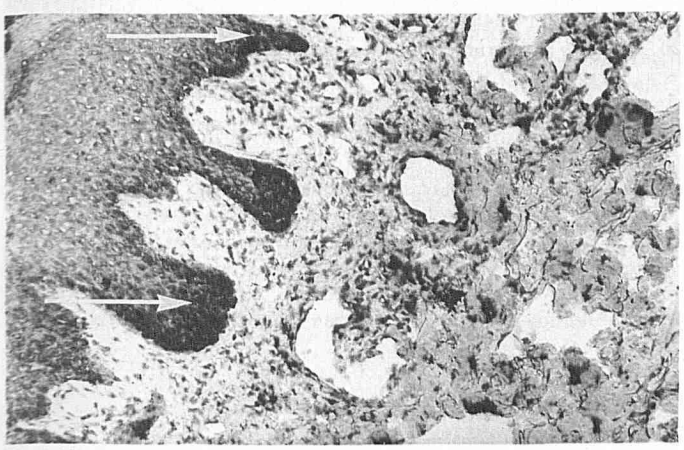

Figure 2. Detection of bFGF in AIDS-KS. Conspicuous expression is found in keratinocytes *(arrows)*. Endothelial and spindle cells do not reveal any detectable amounts of bFGF (magnification ×95).

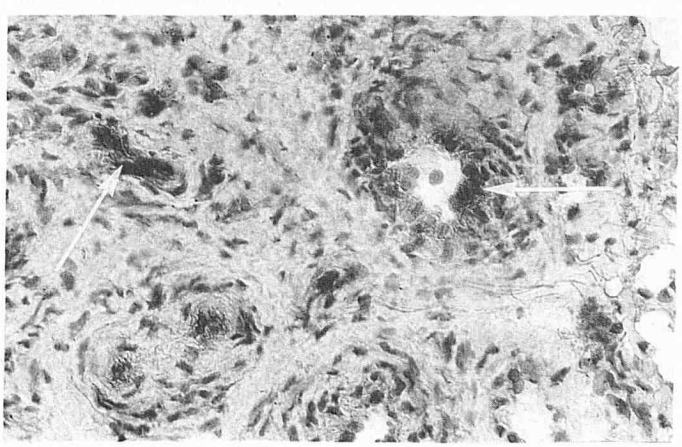

Figure 4. Positive staining of bFGF expression in endothelial cells *(arrows)* of capillary hemangioma (magnification ×240).

Therefore, pronounced expression of bFGF in cultured KS cells may not necessarily be correlated to in vivo situations.

Recently published studies in which skin-derived KS cell lines were analyzed by Northern blotting with various FGF- and FGF-related gene probes also failed to demonstrate elevated bFGF-expression [14]. These data are in line with our in vivo findings. Differences from results obtained by Ensoli et al [10] may be due to a generally observed discrepancy between cellular phenotypes under in vivo and in vitro conditions. Additionally, also the differences in the origin of the analyzed cell types may contribute to the different results, as Ensoli et al [10] used cell lines derived from lung biopsies and pleural effusions [15].

There are, however, several reasons making it unlikely that bFGF-expression is the cause of the abnormal proliferation of Kaposi's sarcoma cells. Basic FGF is lacking a classical hydrophobic signal sequence responsible for external secretion of the growth factor [16]. So the mechanism by which bFGF might be released is totally unknown yet. Rogelj et al [17] observed that transformation of transfected cells only occurred with a bFGF-construct that contained the IgG signal sequence fused 5' to the bFGF coding sequence. Transfections with constructs coding only for bFGF yielded no transformants. Other investigators found transformation of cell lines with extremely high concentrations of the growth factor that would, however, hardly be expressed by tumor cells [18]. The transforming potential of another recently described member of the FGF family exhibiting a typical signal sequence like hst/k-fgf [8] was shown to be 10 to 100 times higher in comparison to bFGF. It will be interesting to investigate whether members of the FGF family such as hst/k-fgf or FGF-5 [19] or still uncharacterized FGF [14] might be involved in KS lesions.

It is notable that other authors did not observe an autocrine growth effect of bFGF on KS cells but a strong dependence of these cells on platelet-derived growth factor [20]. Externally supplied platelet-derived growth factor may be secreted by endothelial cells or extravasated platelets of the highly vacularized tumor. Therefore it can be suggested that paracrine rather than autocrine mechanisms may be the cause for proliferation of KS cells. This is in accordance with the relatively low malignancy and inability of KS cells to grow in nude mice [5].

Taken together, our data and other data make it unlikely that bFGF is the primary agent participating in the etiology of Kaposi's sarcoma. The fact that the growth factor is stored in large amounts in keratinocyes and extracellular matrix [21] suggests a repair-mediated role whereby bFGF is only released upon tissue injury and cell lysis.

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